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BIOMAT 2005**

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Mathematical Modeling of the HIF-1 Mediated Hypoxic Response in Tumours

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Abstract Solid tumours frequently display areas of low oxygen concentration (hypoxia) due to their uncontrolled proliferation and the fact that the new blood vessels they develop are irregular and have poor blood flow. The capacity for a tumour to grow therefore crucially depends on its ability to adjust to hypoxic conditions. It is frequently observed that the hypoxia response pathways, such as angiogenesis (formation of new blood vessels) and glycolysis (use of glucose, rather than oxygen, metabolism), are adapted in tumour cells to allow for aggressive growth in hypoxic conditions. The response pathways are also often upregulated in normoxic conditions. The transcription factor Hypoxia-Inducible Factor 1 (HIF-1) has been found to control the expression of a battery of genes that are crucially involved in the hypoxic response, including key angiogenic growth factors and glycolytic enzymes. Intratumoural hypoxia and HIF-1 overexpression are both associated with poor patient prognosis. In this paper, we extend an ordinary differential equation (ODE) model by Kohn *et al*, *Mol. Biol. of the Cell*, 15:3042 (2004), that measures HIF-1 mediated transcription activation as a function

of oxygen concentration [1]. The model considers a core subsystem of elements from the HIF-1 regulatory network, and in so doing, highlights the stabilisation pathway of the oxygen-regulated HIF-1 α subunit. In normoxic conditions HIF-1 α undergoes a post-translational modification known as hydroxylation, which allows HIF-1 α to be targeted for degradation. In hypoxic conditions, the hydroxylation reaction does not occur, leading to stabilisation of the HIF-1 α protein, formation of the HIF-1 complex and activation of gene transcription. We extend the Kohn model by including mechanisms that may account for the rapid attenuation of the hypoxic response upon reoxygenation of cells, after a period of hypoxia. Our results show good qualitative agreement with experimentally obtained hypoxia dose-response curves by capturing all the important characteristics of the curve.

Keywords Hypoxia; Transcription factor; HIF-1; ODE model; Model reduction

1 Introduction

Owing to genetic and phenotypic diversity, it is increasingly difficult to characterise a typical tumour, or tumour cell. However, a handful of commonly occurring features have been recognised in most tumours. Amongst these, hypoxia is a near universal feature of solid tumour growth. Hypoxia is a reduction in the normal level of tissue oxygen tension and occurs in many circumstances such as acute or chronic vascular disease, pulmonary disease and cancer (for review, see [2]). Cells undergo a variety of adaptive changes in response to hypoxia that are mediated by altered patterns of gene expression in hypoxic areas. The earliest recognised change was observed in cellular metabolism, with increased glycolytic enzyme synthesis leading to a shift to glucose metabolism [3]. Other responses include the induction of erythropoietin to increase haemoglobin production and the widely studied secretion of growth factors by hypoxic cells that induce angiogenesis.

Tumour cells are prone to hypoxia because of the combination of their uncontrolled proliferation and the fact that

the new blood vessels they develop are irregular and have poor blood flow. As a consequence, the capacity for a tumour to progress crucially depends on its ability to adjust to hypoxic conditions. It is thought that tumour cells undergo genetic changes and adapt the hypoxia response pathways in a way that allows them to survive and even proliferate in hypoxic areas. As a result, rather than hindering growth as one may intuitively expect, intratumoral hypoxia is associated with poor prognosis in cancer patients and is an indicator of aggressive growth, increased metastasis and resistance to treatment [4, 5, 6].

1.1 HIF-1

Over the last decade, major advances have been made in understanding the regulation of changes in gene expression triggered by hypoxia. This has been led by the identification of the transcription factor hypoxia inducible factor-1 (HIF-1) [7]. HIF-1 has been found to control the expression of a battery of genes that are crucially involved in the hypoxic response and has subsequently been named a master regulator of oxygen homeostasis [8]. In Figure 1 we highlight five of the main pathways that are either up-regulated or down-regulated in response to hypoxia. More specifically, these are pathways that are frequently manipulated in tumour cells and that we believe are crucial to tumour growth and progression. We illustrate the involvement of HIF-1 by selecting one or two gene products that are involved in activating each of the pathways and whose expression is mediated by HIF-1.

HIF-1 is overexpressed in most common cancers. Overexpression of HIF-1 is believed to be involved in initiating responses such as angiogenesis and glycolysis even under normoxic conditions. Understanding the effects of hypoxia, and more specifically, the HIF pathway, may therefore help elucidate the mechanisms underlying cancer progression that are shared by most or perhaps all types of human cancer. Both hypoxia and increased HIF-1 expression are associated with poor patient prognosis and may provide an important therapeutic target. We believe the hypoxia response is a key driver in the behaviour of cancer and that HIF-1 lies crucially at the

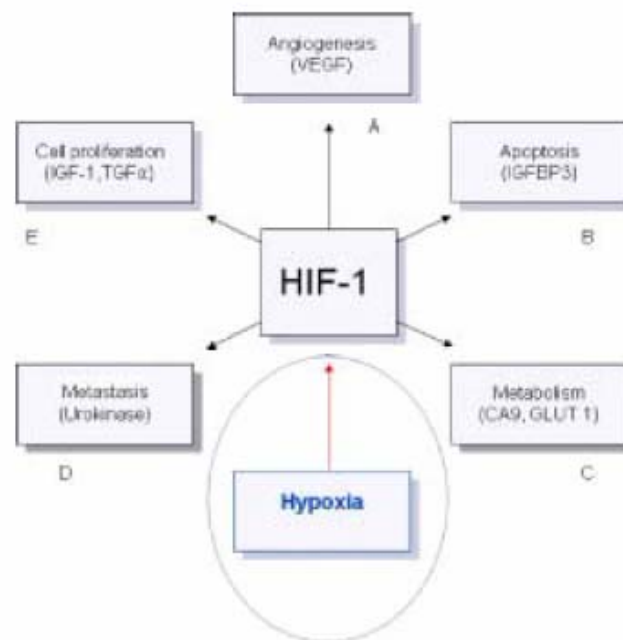


Figure 1: Schematic diagram to illustrate the involvement of HIF-1 in the hypoxic response. (A) Vascular endothelial growth factor (VEGF) has been shown to be a potent inducer of angiogenesis [9]. (B) Insulin-like growth factor binding protein (IGFBP)-3 functions as a proapoptotic agent by binding free insulin-like growth factor (IGF)-1 and 2, thereby preventing these ligands from stimulating the type-1 receptor (IGF-1R). IGF-1R is responsible for inducing mitosis and protecting against apoptosis, and plays a role in cellular transformation [10]. (C) Glucose transporter (GLUT)-1 expression leads to an enhanced glucose flux across the cell membrane [3] and carbonic anhydrase (CA) 9 provides a mechanism for maintaining the acidic extracellular microenvironment that is characteristic of tumours [11]. Both these proteins have therefore been implicated in promoting the glycolytic phenotype in tumour cells. (D) Metastasis, the movement of cells from the primary tumour to distant parts of the body, results from a combination of complex processes such as cell proliferation, angiogenesis, degradation of the extracellular matrix (ECM) and decreased cell-adhesion. The urokinase-type plasminogen activator system is inextricably linked with a number of these processes [12]. (E) Transforming growth factor (TGF)- α and IGF-1 are two growth factors that can stimulate cell proliferation in hypoxic conditions.

heart of the response pathway. In this paper we therefore model the induction of HIF-1 in conditions of low oxygen concentration. In other words we model the 'Hypoxia to HIF-1' arrow circled in Figure 1, with the aim of eventually incorporating a caricature version into a model of the 'bigger picture' as shown by the rest of the figure.

We now briefly present some background on the HIF-1 transcription factor and its regulation. To cover all of the biological details concerning the regulation of HIF-1 is beyond the scope of this paper. We only present facts that are directly relevant to our mathematical model and refer the reader to [13] for a comprehensive review. In Section 3 we present our mathematical model to describe the induction of HIF-1 and in Section 4 perform some preliminary analysis of our model. We present the results of our model in Section 5 and end in Section 6 with a discussion.

2 Background Biology

HIF-1 is a heterodimer that consists of HIF-1 α and HIF-1 β subunits, which are basic helix-loop-helix - PAS domain proteins [14]. HIF-1 β is an obligatory but constitutively expressed component of HIF-1. It had already been identified as aryl hydrocarbon receptor nuclear translocator (ARNT), the dimerization partner of aryl hydrocarbon receptor (AHR) [15]. HIF-1 β can, in fact, dimerize with several different bHLH-PAS proteins, including the related HIF-2 α protein, whereas HIF-1 α is the unique, oxygen regulated sub-unit that determines HIF-1 activity.

As with any protein, the steady state levels of HIF-1 α protein are regulated at the level of synthesis and degradation. The expression of HIF-1 α is maintained at low levels in most cells under normoxic conditions and is constantly synthesized and degraded to ensure a rapid response to hypoxia. In the case of HIF-1 α , synthesis is regulated in an oxygen independent manner, whereas degradation is regulated via oxygen dependent mechanisms [16], as shown in Figure 2. In Figure 2 we have essentially zoomed in on Figure 1 to give a second, more detailed, schematic interpretation of the HIF mediated

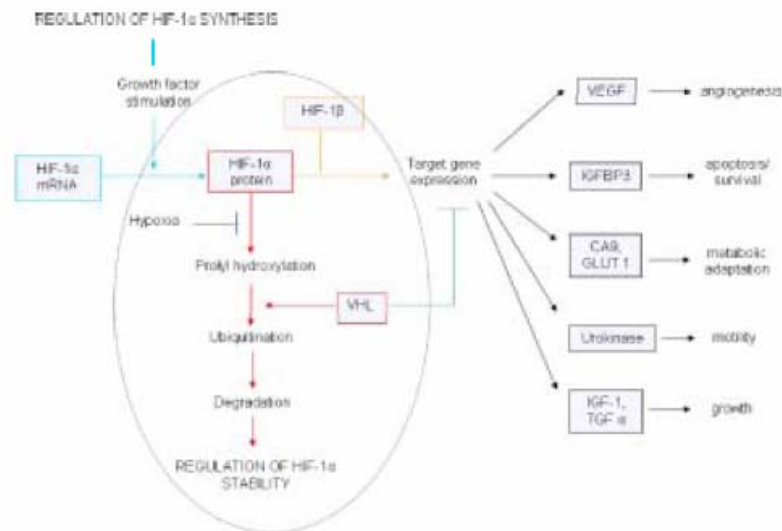


Figure 2: Detailed schematic diagram to illustrate the involvement of HIF-1 in the hypoxic response. Adapted from [17].

hypoxic response. Particular emphasis has been placed on the stabilisation pathway because this is the pathway that relates directly to the 'Hypoxia to HIF-1' arrow that were are modeling, as highlighted in Figure 1. The details of the stabilisation pathway will be given in more detail later.

2.1 Synthesis

HIF-1 α protein synthesis is induced by growth factor stimulation via activation of one of two signalling cascades, PI3K/Akt or MAPK. In particular, epidermal growth factor receptor (EGFR), insulin like growth factor (IGF) and human epidermal growth factor 2 (HER2) have been implicated in activating the above pathways [18, 19, 20]. Transforming growth factor α (TGIF α) belongs to a family of ligands that bind, and hence activate, one or several members of the EGFR family. The result of growth factor stimulation is an increase in the rate at which a subset of mRNAs (including HIF-1 α mRNA) is translated into protein [16]. TGIF α (as well as EGFR and IGF-1) has been shown to be up-regulated by HIF-1, thereby forming a posi-

tive feedback loop. The PI3K pathway is negatively regulated by the tumour suppressor protein PTEN.

2.2 Stabilisation

In the presence of oxygen, three prolyl hydroxylases have been shown to post-transcriptionally modify HIF-1 α . The three enzymes, named prolyl hydroxylase domain containing proteins 1,2 and 3 (PHD1,2 and 3), use oxygen as a substrate to generate 4-hydroxyproline at the proline residues Pro⁴⁰² and/or Pro⁵⁶⁴, thereby hydroxylating at least one of these two critical residues [21] under normoxic conditions. Following the hydroxylation reaction, HIF-1 is able to interact with the tumour suppressor von-Hippel-Lindau (VHL) which instigates the ubiquitination of HIF-1 α .

Ubiquitination (also known as ubiquitylation and ubiquitynylation) refers to the formation of a multiubiquitin chain on the protein so that it can be targeted for degradation by the proteasome. Ubiquitinated HIF-1 α is targeted for degradation by the proteasome. Thus, under normoxic conditions, HIF-1 α is rapidly degraded and has a half-life of less than 5 min, both *in vivo* and *in vitro* [14, 22].

Conversely, under hypoxic conditions, prolyl hydroxylase cannot modify HIF-1 α ; subsequently VHL cannot bind HIF-1 α thus ubiquitination is greatly reduced and the protein remains stable. Stabilised HIF-1 α is translocated to the nucleus where it interacts with HIF-1 β to form the HIF-1 complex [2]. PHD2 and 3, but not PHD1, are upregulated by the HIF-1 transcription factor, giving rise to a negative feedback loop.

2.3 DNA binding and gene transcription

Once in the nucleus, as well as dimerization with HIF-1 β to form HIF-1, HIF-1 α interacts with co-factors such as CBP and p300 and the DNA polymerase II (Pol II) complex. We do not consider co-factors in our mathematical model however it is only in this state that HIF-1 is able to bind to a specific DNA sequence named hypoxia-responsive element (HRE) and activate the transcription of HIF-1 target genes [23].

Hypoxia-responsive elements (HREs) are DNA sequences

of 50 base pairs or less that contain functionally essential HIF-1 binding sites of the consensus sequence 5'-RCGTG-3' [24]. HREs were first identified in genes encoding EPO and have now also been identified in promoter regions of human and mouse genes encoding VEGF, glycolytic enzymes and glucose transporters, amongst others [25, 26].

2.4 Gene transcription repression

We explained above in Section 2.2 how HIF-1 α stability is regulated by oxygen concentration and in Section 2.3 how stabilised HIF-1 α can bind to DNA. Interestingly, a mechanism involving oxygen-dependent hydroxylation, similar to that controlling the stability of the HIF-1 α protein, has been found to regulate HIF-mediated gene transcription. Although we do not model this second regulatory mechanism we feel that it is important to mention it here. It was shown that under normoxic conditions HIF-1 α is subject to a second bout of hydroxylation, in this case at the asparagine residue Asp⁸⁰³ within the C-terminal transactivation domain (TAD-C). Asparagine hydroxylation is performed by Factor Inhibiting HIF-1 (FIH-1) and prevents the interaction of HIF-1 α with p300. As a result, the HIF complex may bind to HREs but is unable to activate gene transcription. In other words, competitive binding at the TAD-C between p300 and FIH-1 provides another 'brake' on HIF function when oxygen is not limiting.

2.5 HIF-1 overexpression

The expression of HIF-1 α is markedly increased in most common human cancers and their metastases [27, 28]. Significant associations between HIF-1 α over-expression and patient mortality have been shown in certain cancers. The increased levels of HIF-1 α can be attributed in part to tumour hypoxia and growth factor stimulation (as described above). In addition, there are a number of mutations that have been reported to affect HIF-1 α levels, such as loss of PTEN [20, 29], and p53 [30]. The most famous is the loss-of-function of the VHL [31] tumour suppressor gene.

Germline mutations of VHL cause a hereditary cancer syn-

drome called von Hippel-Lindau disease. The disease is associated with the development of certain tumours, including hemangioblastomas of the retina and central nervous system, clear cell renal carcinomas, and pheochromocytomas [32]. These tumours express constitutive levels of HIF-1 α protein and HIF-1 target products such as VEGF, resulting in highly vascularised tumours.

3 Mathematical Model

3.1 Previous Mathematical Models

With respect to theoretical models, this area is almost untouched. The only previous theoretical model to address HIF induction has been recently published by Kohn and colleagues [1]. Kohn *et al* formulated a molecular interaction map to describe the hypoxia-induced transcriptional response. Molecular interaction maps (MIMs) were introduced by Kohn [33] as a diagrammatic convention that is capable of unambiguous representation of gene regulatory networks. The hypoxia MIM incorporated all known biological details of HIF regulation from scientific literature at the time. As a consequence the full MIM is extremely large and complicated. The full hypoxia MIM can also be found on Kohn's group website (<http://discover.nci.nih.gov>) where it is frequently updated as new discoveries are made. However, in order to minimise the complexity of the model, Kohn *et al* isolated a smaller core subsystem from the full MIM. The core subsystem consisted of HIF-1 α , HIF-1 β , PHD 2 and 3, VHL and Hypoxia Response Elements (HREs). The model interactions are based on the biological detail presented above.

Kohn *et al* used a computer program to translate the interactions described in the molecular map into a set of ordinary differential equations based on the law of mass action, and then used an iterative finite difference method to approximate the solution. The rate constants were found using a search of the parameter space that gave the steepest switch-like response of the output species (HRE occupancy) as a function of the input species (oxygen).

A hypoxia dose-response curve published by Jiang *et al* [34]

showed that HIF-1 is induced rapidly as oxygen tension decreases from 2% to 0.5% oxygen. In Figure 3(a) we have produced a schematic representation of the dose-response curve obtained by Jiang *et al.* There is an almost switch-like transition. Surprisingly, however, when a further set of experiments was performed between 0% and 2% oxygen, HIF-1 α protein levels and HIF-1 DNA activity showed a maximal response at 0.5% oxygen, with the response diminishing thereafter as oxygen approached 0% (as seen in the inset of Figure 3(a)).

Although huge advances have been made in the understanding of the HIF-1 regulation and various explanations have been suggested [35], no generally agreed framework has been accepted. The most widely accepted is the prolyl/asparagine hydroxylation model we described above but this does not seem to account for the decreasing response between 0.5 and 0% oxygen. As Schumacker explains, this model predicts that complete anoxia should inhibit hydroxylation of HIF-1 α since O₂ is required as a substrate [36]. He therefore questions whether multiple oxygen sensors exist within one cell, acting at different ranges of oxygen tension. Similarly, Semenza observes that the plot of HIF-1 α yields a sigmoidal curve suggestive of cooperativity, but this finding is not readily explained by the known biochemistry of the HIF-1 α prolyl hydroxylases [37].

Kohn *et al* [1] manage to capture the switch-like induction of HIF with decreasing oxygen tension. However as oxygen concentration decreases further towards 0% they predict a plateau response rather than one that is obviously decreasing. Figure 3(b) shows a schematic representation of the result from the Kohn model. We aim to develop a theoretical model that can capture both important features of the curve in Figure 3(a), thereby suggesting mechanisms that give rise to the observed behaviour. In the next section we present our mathematical model which is an extension of the Kohn model. Our model follows the Kohn model unless otherwise specified.

3.2 Model equations

In this section we propose a hypoxia response model that involves two-compartment kinetics of the HIF-1 α protein. The

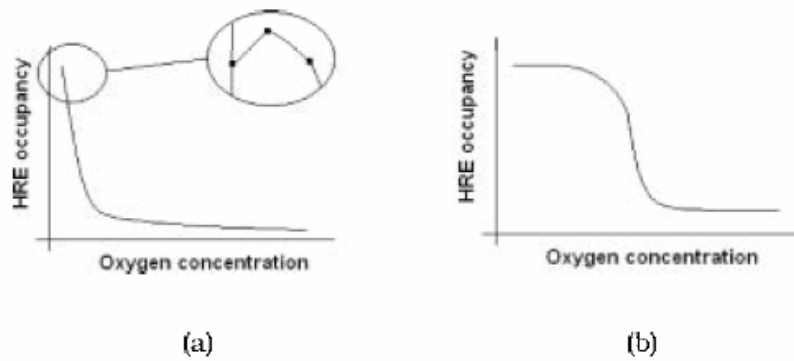


Figure 3: Schematic diagram to illustrate the hypoxia dose-response curve obtained (a) experimentally, by Jiang *et al* [34], and (b) theoretically, by Kohn *et al* [1].

model by Kohn *et al* is a single compartment model so we have extended it by considering cytoplasmic and nuclear compartments. In the cytoplasm we assume that HIF-1 α is hydroxylated by a generic cytoplasmic prolyl hydroxylase domain containing protein ($PHD_c(t)$), and that hydroxylated HIF-1 α , denoted by $H_{\alpha P}(t)$, is bound by VHL for ubiquitination and degradation. We note here that in reality it is a VHL complex, including other proteins and cofactors, and not VHL alone, that targets HIF-1 α for ubiquitination [32]. However, we assume this complex is a single protein denoted by $VHL(t)$ and we do not consider the dynamics leading to the complex formation.

We assume that unhydroxylated HIF-1 α becomes stable and is translocated to the nucleus using the transport equations. Hence HIF-1 α can exist in two forms, cytoplasmic HIF-1 α (denoted by $H_{\alpha}(t)$) and stabilised or nuclear HIF-1 α (denoted by $H_{\alpha N}(t)$).

In our model, stabilised HIF-1 α can dimerize with HIF-1 β protein ($H_{\beta}(t)$) to form the HIF-1 complex, $H_1(t)$. The HIF-1 complex can then bind to Hypoxia Response Elements ($HRE(t)$) which would subsequently initiate the transcription of HIF-1 target genes. Note here that we have omitted the hydroxylation of HIF-1 α in the asparagine residue via FIH-1. In other words we only consider the regulation of HIF-1 α sta-

bility and ignore the secondary mechanism of HIF transcriptional regulation. We therefore follow the convention used in Kohn *et al* [1] and use *occupied HRE* (i.e HRE bound by HIF-1) as a measure of HIF-1 transcriptional activity. We denote occupied HRE by $HRE'(t)$.

Similarly, we assume the hydroxylated HIF-1 α that escapes the ubiquitination and degradation pathway can be translocated to the nucleus, to give a stable form of hydroxylated HIF-1 α which is denoted by $H_{\alpha NP}(t)$. Binding with the HIF-1 β protein and Hypoxic Response Elements gives hydroxylated HIF-1, $H_{1P}(t)$, and occupied HRE (hydroxylated), $HRE^*(t)$, respectively.

Additionally, we assume that hydroxylation and ubiquitination can occur in the nuclear compartment [38, 39]. Proline hydroxylase domain containing protein in the nucleus ($PHD_n(t)$) can hydroxylate nuclear HIF-1 α such that it takes the form of nuclear hydroxylated HIF-1 α , $H_{\alpha NP}(t)$. Nuclear hydroxylated HIF-1 α can be bound by VHL to form the ($H_{\alpha NP} : VHL_n$) complex and is ubiquitinated and degraded thereafter.

One major way in which our model differs from the Kohn model is that we assume the dissociation of HIF-1 complex, as well as HIF-1 bound to HRE, is linearly dependent on oxygen. Since we are considering a two compartment model we also assume that nuclear export of HIF-1 α is oxygen-dependent. These assumptions are made on the following basis: the inhibition of prolyl hydroxylation under hypoxic conditions has been identified as the mechanism that allows for the rapid nuclear accumulation of HIF-1 α . However, a rapid response to *reoxygenation* has also been observed [40]. Hypoxia-induced HIF-1 binding activity as well as HIF-1 α protein levels were reported to be rapidly and drastically decreased *in vivo* following an abrupt increase in normal oxygen tension. HIF-1 α is degraded with a half-life close to 90 seconds upon reoxygenation of hypoxic HeLa cells. Furthermore, another report states that although HIF-1 α localises exclusively in the nucleus of hypoxic cells, it is exported to the cytoplasm upon reoxygenation [38] to undergo protein degradation.

Other more subtle differences in the two models are listed below:

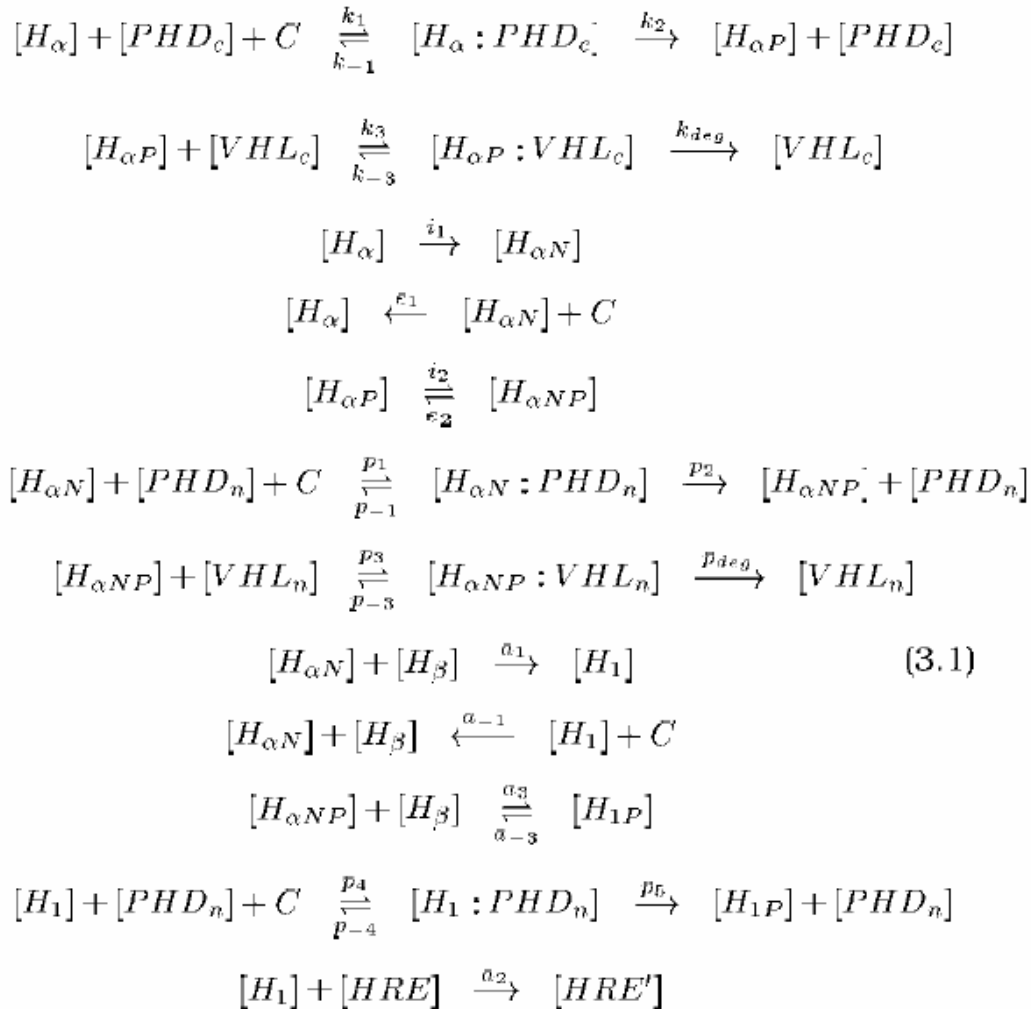
- we assume HIF-1 α and PHD bind in an oxygen depen-

dent manner and then dissociate to produce hydroxylated HIF-1 α in an oxygen independent manner

- we assume there is some removal/degradation of occupied HRE. That is, we assume that there is some additional degradation term for HIF-1 α after participation in gene transcription.

In addition we consider the synthesis, degradation and translation of all the protein mRNA, i.e HIF-1 α , HIF-1 β , PHD and VHL mRNA transcript, for completeness.

We have included below the kinetic equations on which our model is based, Equations (3.1), and a schematic diagram of our model interactions is given in Figure 4.



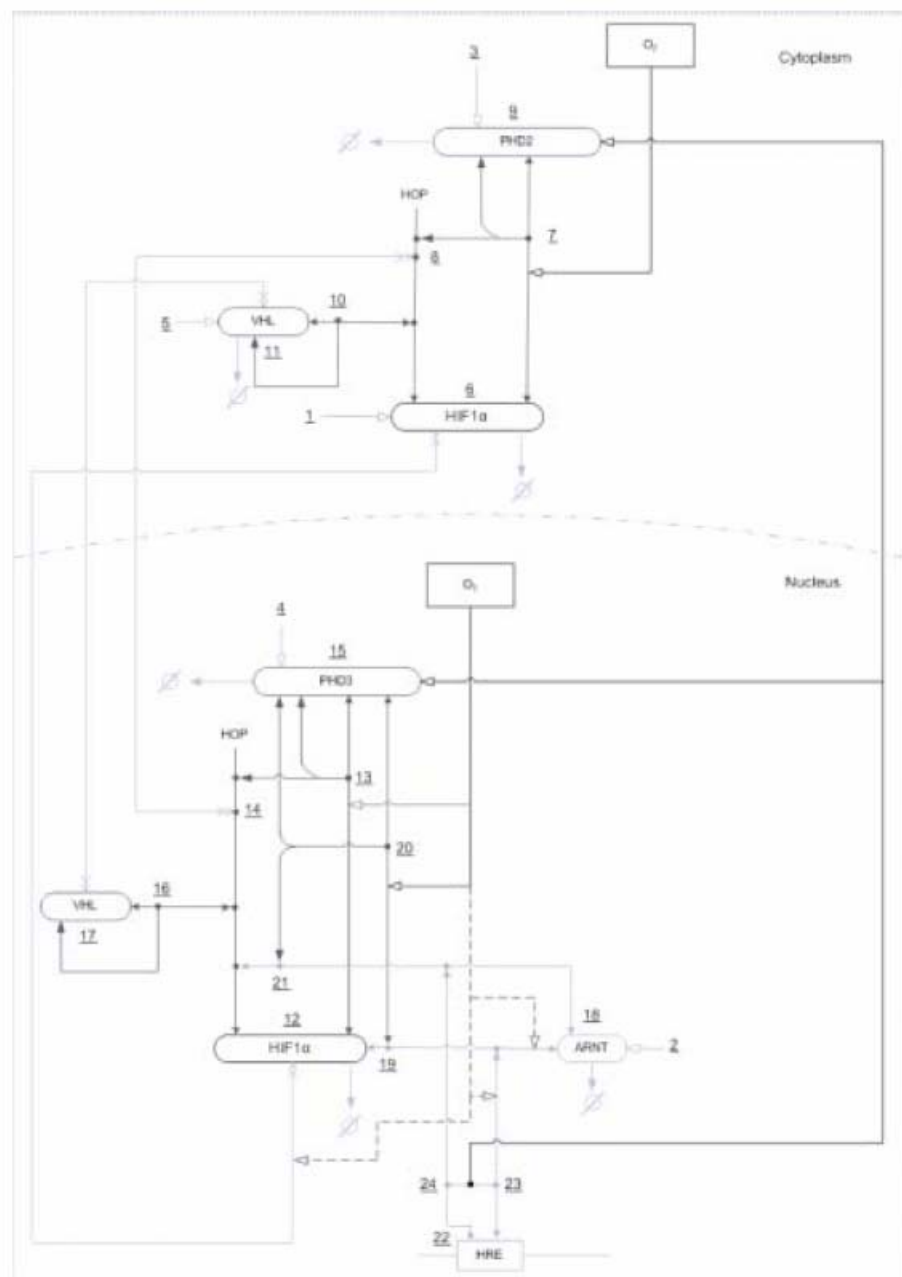
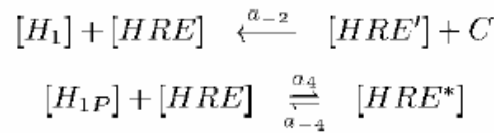


Figure 4: MIM relating to our full model, Equations (3.2) - (3.26).



We now present our model equations and discuss each one in turn. We employ a system of ODEs and the law of mass action to describe the kinetics of our substrates and complexes. A list of the constant parameters in our equations below can be found in Table 2.

HIF-1 α and HIF-1 β transcript (x_1, x_2):

$$\frac{dx_1}{dt} = c_1 - c_2 x_1 \quad (3.2)$$

$$\frac{dx_2}{dt} = c_5 - c_6 x_2. \quad (3.3)$$

These equations represent, respective, synthesis of HIF-1 α and HIF-1 β mRNA, and degradation of the transcript.

Cytoplasmic and nuclear PHD transcript (x_3, x_4):

$$\frac{dx_3}{dt} = c_{c9} + c_{c10} x_{22} - c_{c11} x_3 \quad (3.4)$$

$$\frac{dx_4}{dt} = c_{n9} + c_{n10} x_{22} - c_{n11} x_4. \quad (3.5)$$

These equations model constitutive synthesis, HIF-inducible synthesis and degradation of PHD mRNA.

VHL transcript (x_5):

$$\frac{dx_5}{dt} = c_{14} - c_{15} x_5; \quad (3.6)$$

represents constitutive VHL mRNA synthesis and degradation.

Free cytoplasmic HIF-1 α protein (x_6):

$$\frac{dx_6}{dt} = c_3 x_1 - c_4 x_6 - k_1(C) x_6 x_9 + k_{-1} x_7 - i_1 x_6 + e_1(C) x_{13}. \quad (3.7)$$

describes translation of HIF-1 α mRNA transcript into protein, spontaneous protein degradation, formation and dissociation of the (HIF-1 α :PHDc) complex, where the formation is assumed to be dependent on oxygen concentration C , and cellular transport. Note that nuclear export is also assumed to be oxygen-dependent.

Cytoplasmic (HIF-1 α :PHDc) complex (x_7):

$$\frac{dx_7}{dt} = k_1(C)x_6x_9 - k_{-1}x_7 - k_2x_7. \quad (3.8)$$

This equation represents the assumed to be oxygen-dependent association of HIF-1 α and PHDc, spontaneous dissociation of the complex, and dissociation due to the formation of the hydroxylated HIF-1 α product.

Hydroxylated HIF-1 α (x_8):

$$\frac{dx_8}{dt} = k_2x_7 - c_4x_8 - k_3x_8x_{11} + k_{-3}x_{10} - i_2x_8 + e_2x_{14}; \quad (3.9)$$

describes production of hydroxylated HIF-1 α from the (HIF-1 α :PHDc) complex, spontaneous degradation, formation and dissociation of the (Hydroxylated HIF-1 α :VHL) complex, and cellular transport.

PHDc protein (x_9):

$$\frac{dx_9}{dt} = c_{c12}x_3 - c_{c13}x_9 - \quad (3.10)$$

$$k_1(C)x_6x_9 + k_{-1}x_7 + k_2x_7; \quad (3.11)$$

accounts for mRNA translation, spontaneous protein degradation, formation and dissociation of the (HIF-1 α :PHDc) complex, where the formation is assumed to be dependent on oxygen, and dissociation due to the formation of the hydroxylated HIF-1 α product.

Hydroxylated HIF-1 α - VHL complex (x_{10}):

$$\frac{dx_{10}}{dt} = k_3x_8x_{11} - k_{-3}x_{10} - k_{deg}x_{10}. \quad (3.12)$$

This equation models the formation and dissociation of the (Hydroxylated HIF-1 α :VHL) complex, and loss of hydroxylated HIF-1 α due to ubiquitination and degradation.

Cytoplasmic VHL (x_{11}):

$$\frac{dx_{11}}{dt} = c_{16}x_5 - c_{17}x_{11} - k_3x_8x_{11} + k_{-3}x_{10} + k_{deg}x_{10} - i_4x_{11} + e_4x_{17}; \quad (3.13)$$

represents translation of VHL transcript, spontaneous degradation of VHL protein, and formation and dissociation of the (hydroxylated HIF-1 α :VHL) complex. Also describes the dissociation of hydroxylated HIF-1 α due to ubiquitination and degradation, and constitutive cellular transport.

Free Nuclear HIF-1 α (x_{12}):

$$\begin{aligned} \frac{dx_{12}}{dt} = & i_1k_vx_6 - e_1k_v(C)x_{13} - k_{deg2}x_{12} - p_1(C)x_{12}x_{15} + p_{-1}x_{13} \\ & - a_1x_{12}x_{18} + a_{-1}(C)x_{19}; \end{aligned} \quad (3.14)$$

the first two terms describe the transport into and out of the nucleus, where export is assumed to be dependent on oxygen. Both terms have been adjusted to the nuclear volume by the coefficient $k_v = V/U$. The remaining terms represent spontaneous degradation of HIF-1 α , formation and dissociation of the (HIF-1 α :PHDn) complex, where the formation is assumed to be dependent on oxygen concentration, and the formation and (assumed) oxygen-dependent dissociation of the HIF-1 complex.

Nuclear HIF-1 α - PHDn complex (x_{13}):

$$\frac{dx_{13}}{dt} = p_1(C)x_{12}x_{15} - p_{-1}x_{13} - p_2x_{13}. \quad (3.15)$$

Represents the association and dissociation of HIF-1 α and PHDn, and dissociation due to the formation of the nuclear hydroxylated HIF-1 α product.

Nuclear hydroxylated HIF-1 α (x_{14}):

$$\begin{aligned} \frac{dx_{14}}{dt} = & i_2 k_v x_8 - e_2 k_v x_{14} - k_{deg2} x_{14} + p_2 x_{13} - p_3 x_{14} x_{17} + p_{-3} x_{16} \\ & - a_3 x_{14} x_{18} + a_{-3} x_{21}. \end{aligned} \quad (3.16)$$

Describes cellular transport, and spontaneous degradation of the hydroxylated HIF-1 α protein. Additionally models production of hydroxylated HIF-1 α from the nuclear (HIF-1 α : PHDn) complex, formation and dissociation of the (Hydroxylated HIF-1 α :VHL) complex, and formation and dissociation of the hydroxylated HIF-1 complex.

PHDn protein (x_{15}):

$$\begin{aligned} \frac{dx_{15}}{dt} = & c_{n12} x_4 - c_{n13} x_{15} - p_1(C) x_{12} x_{15} + p_{-1} x_{13} + p_2 x_{13} \\ & - p_4(C) x_{19} x_{15} + p_{-4} x_{20} + p_5 x_{20}. \end{aligned} \quad (3.17)$$

Describes translation of PHDn mRNA into protein and spontaneous protein degradation. The remaining terms represent the formation and dissociation of the (HIF-1 α :PHDn) and (HIF-1:PHDn) complexes, and dissociation due to the formation of the hydroxylated HIF-1 α product and hydroxylated HIF-1 product, respectively.

Nuclear Hydroxylated HIF-1 α - VHL complex (x_{16}):

$$\frac{dx_{16}}{dt} = p_3 x_{14} x_{17} - p_{-3} x_{16} - p_{deg} x_{16}. \quad (3.18)$$

Describes the formation and dissociation of the (hydroxylated HIF-1 α :VHL) complex, and the loss due to ubiquitination and degradation of nuclear hydroxylated HIF-1 α .

Nuclear VHL (x_{17}):

$$\begin{aligned}\frac{dx_{17}}{dt} = & -p_3x_{14}x_{17} + p_{-3}x_{16} + p_{deg}x_{16} + i_Ak_vx_{11} \\ & -e_Ak_vx_{17}.\end{aligned}\quad (3.19)$$

Describes the formation and dissociation of the (hydroxylated HIF-1 α :VHL) complex, dissociation due to ubiquitination and degradation of hydroxylated HIF-1 α , and nuclear-cytoplasmic shuttling of the VHL protein.

HIF-1 β protein (x_{18}):

$$\begin{aligned}\frac{dx_{18}}{dt} = & c_7x_2 - c_8x_{18} - a_1x_{12}x_{18} + a_{-1}(C)x_{19} \\ & -a_3x_{14}x_{18} + a_{-3}x_{21}.\end{aligned}\quad (3.20)$$

Describes translation of HIF-1 β mRNA transcript, spontaneous protein degradation, and formation and dissociation of the HIF-1 and hydroxylated HIF-1 complexes. Dissociation is assumed to be oxygen-dependent in the non-hydroxylated case.

HIF-1 complex (x_{19}):

$$\begin{aligned}\frac{dx_{19}}{dt} = & a_1x_{12}x_{18} - a_{-1}(C)x_{19} - a_2x_{19}x_{22} + a_{-2}(C)x_{23} \\ & -p_4(C)x_{19}x_{15} + p_{-4}x_{20}.\end{aligned}\quad (3.21)$$

This equation represents the formation and (assumed) oxygen-dependent dissociation of the HIF-1 complex, the binding of HIF-1 to HREs and (assumed) oxygen-dependent dissociation, and the binding and dissociation of HIF-1 and PHDn.

HIF-1 - PHDn complex (x_{20}):

$$\frac{dx_{20}}{dt} = p_4(C)x_{19}x_{15} - p_{-4}x_{20} - p_5x_{20}.\quad (3.22)$$

Represents the assumed to be oxygen-dependent association of HIF-1 and PHDn, the dissociation of the complex, and the

dissociation due to the formation of the hydroxylated HIF-1 product.

Hydroxylated HIF-1 complex (x_{21}):

$$\begin{aligned} \frac{dx_{21}}{dt} = & p_5 x_{20} + a_3 x_{14} x_{18} \\ & - a_{-3} x_{21} - a_4 x_{21} x_{22} + a_{-4} x_{24}. \end{aligned} \quad (3.23)$$

Represents the formation of the hydroxylated HIF-1 product, the formation and dissociation of the hydroxylated HIF-1 complex through the binding of hydroxylated HIF-1 α and the HIF-1 β protein, and the binding and dissociation of hydroxylated HIF-1 to HREs.

HRE (x_{22}):

$$\begin{aligned} \frac{dx_{22}}{dt} = & -a_2 x_{19} x_{22} + a_{-2}(C) x_{23} - a_4 x_{21} x_{22} + a_{-4} x_{24} + k_{deg3} x_{23} \\ & + k_{deg3} x_{24}. \end{aligned} \quad (3.24)$$

Represents the binding of HIF-1 and hydroxylated HIF-1 to HREs, where dissociation is assumed to be oxygen-dependent in the non-hydroxylated case. Additionally includes an occupied HRE degradation/removal term.

Non-hydroxylated and hydroxylated Occupied HRE (x_{23} , x_{24}):

$$\frac{dx_{23}(t)}{dt} = a_2 x_{19}(t) x_{22}(t) - a_{-2}(C) x_{23}(t) - k_{deg3} x_{23}(t) \quad (3.25)$$

$$\frac{dx_{24}(t)}{dt} = a_4 x_{21}(t) x_{22}(t) - a_{-4} x_{24}(t) - k_{deg3} x_{24}(t). \quad (3.26)$$

Describes the respective binding of HIF-1 and hydroxylated HIF-1 to HREs, where dissociation is assumed to be oxygen-dependent in the non-hydroxylated case. Additionally includes an occupied HRE degradation/removal term.

Variable Notation		Description	Initial Value (mM)
1	2		
x_1	$H_{\alpha t}$	HIF-1 α transcript	0.0012
x_2	$H_{\beta t}$	HIF-1 β transcript	0.0012
x_3	PHD_{ct}	PHDc transcript	0.0012
x_4	PHD_{nt}	PHDn transcript	0.0012
x_5	VHL_t	VHL transcript	0.0012
x_6	H_{α}	Cytoplasmic HIF-1 α protein	0.1
x_7	$(H_{\alpha} : PHD_c)$	Cytoplasmic (HIF-1 α :PHDc) complex	1
x_8	$H_{\alpha P}$	Cytoplasmic hydroxylated HIF-1 α	0.1
x_9	PHD_c	PHDc protein	1
x_{10}	$(H_{\alpha P} : VHL_c)$	Cytoplasmic (hydroxylated HIF-1 α :VHL)	1
x_{11}	VHL_c	Cytoplasmic VHL	10
x_{12}	$H_{\alpha N}$	Nuclear HIF-1 α protein	0.1
x_{13}	$(H_{\alpha N} : PHD_n)$	Nuclear (HIF-1 α :PHDn) complex	0.1
x_{14}	$H_{\alpha NP}$	Nuclear hydroxylated HIF-1 α	0.1
x_{15}	PHD_n	PHDn protein	1
x_{16}	$(H_{\alpha NP} : VHL_n)$	Nuclear (hydroxylated HIF-1 α :VHL) complex	0.1
x_{17}	VHL_n	Nuclear VHL	5
x_{18}	H_{β}	HIF-1 β protein	0.1
x_{19}	H_1	HIF-1	0.1
x_{20}	$(H_1 : PHD_n)$	(HIF-1:PHDn) complex	0.1
x_{21}	H_{1P}	Hydroxylated HIF-1	0
x_{22}	HRE	HRE	0.8
x_{23}	HRE^i	Occupied HRE	1
x_{24}	HRE^*	Occupied (hydroxylated) HRE	0.1

Table 1: Model variable notation plus initial conditions.

We note that we have hypothesized that certain processes in our model are oxygen-dependent. In other words, the reaction rates for those processes may be a function of the oxygen concentration C . As a first approximation we assume that the dependence is linear.

4 Analysis

As we mentioned earlier, one of the major ways in which we have adapted the Kohn model is by including oxygen-dependent dissociation and nuclear export in certain cases. The reason for this is to take into account the rapid attenuation of the HIF response upon re-oxygenation after a period of hypoxia. We will now use simple mathematical analysis to investigate what effect this may have on our model results.

Let us consider the equations for free cytoplasmic HIF-1 α protein and nuclear HIF-1 α protein. That is equations (3.7)

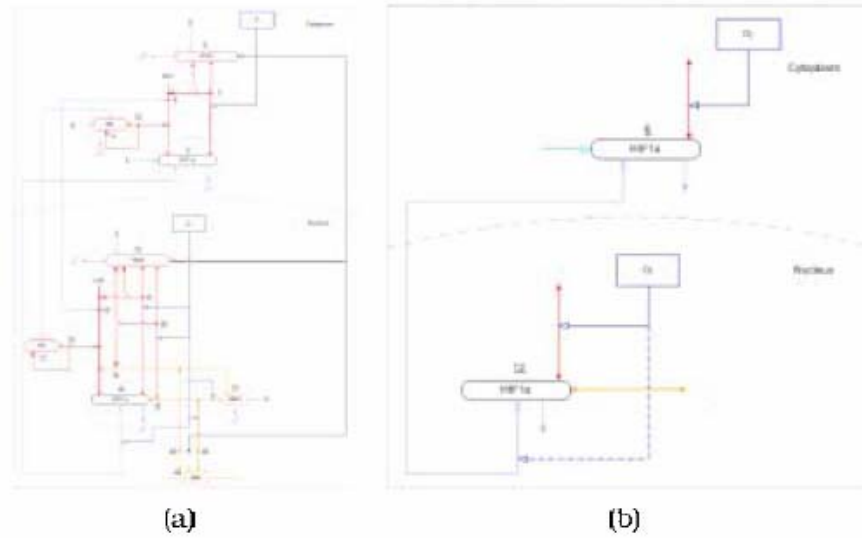


Figure 5: (a) Free cytoplasmic and nuclear HIF-1 α protein highlighted on our model schematic diagram. (b) Isolation of these two species to illustrate simplifications made in Equations (4.1) and (4.2).

and (3.14) for x_6 and x_{12} respectively,

$$\begin{aligned}\frac{dx_6}{dt} &= c_3x_1 - c_4x_6 - k_1(C)x_6x_9 + k_{-1}x_7 - i_1x_6 + e_1(C)x_{12} \\ \frac{dx_{12}}{dt} &= i_1k_vx_6 - e_1k_v(C)x_{12} - k_{deg}x_{12} - p_1(C)x_{12}x_{15} + p_{-1}x_{13} \\ &\quad - a_1x_{12}x_{18} + a_{-1}(C)x_{19}.\end{aligned}$$

In Figure 5(a) we highlight the areas of the schematic diagram of our model on which we are focusing.

Let us ignore the formation of the HIF-1 complex and any further species and assume that $H_{\alpha t}(t)$, $PHD(t)$, $(H_{\alpha} : PHD_c)(t)$ and $(H_{\alpha N} : PHD_n)(t)$ are constant, or equivalently assume x_1, x_7, x_9, x_{13} and x_{15} are constant. We can group like terms and simplify equations (3.7) and (3.14) to the following,

$$\frac{dx_6}{dt} = p_1 - p_2x_6 - p_3Cx_6 + p_4x_{12} \quad (4.1)$$

$$\frac{dx_{12}}{dt} = p_5x_6 - p_4Cx_{12} - p_6x_{12}, \quad (4.2)$$

where the p 's are all constant. Figure 5(b) illustrates the above simplifications. We can now find the steady state of this system as a function of oxygen concentration C :

$$\begin{aligned} \frac{dx_6}{dt} &= 0 \\ \Rightarrow x_6 &= \frac{p_1}{p_2 + p_3 C - \frac{p_4 p_5 C}{p_4 C + p_6}} \end{aligned} \quad (4.3)$$

and

$$\begin{aligned} \frac{dx_{12}}{dt} &= 0 \\ \Rightarrow x_{12} &= \frac{p_1 p_5 / (p_4 C + p_6)}{p_2 + p_3 C - \frac{p_4 p_5 C}{p_4 C + p_6}}. \end{aligned} \quad (4.4)$$

In the first panel of Figure 6 we have plotted the denominator of x_{12} as a function of oxygen tension, where x_{12} is given by Equation (4.4). In the second panel we have plotted x_{12} as a function of oxygen tension and we can see that the concentration of x_{12} ($H_{\alpha N}$) exhibits an “exponential” type increase as the oxygen concentration decreases but peaks before the oxygen concentration reaches zero. This is in keeping with the experimental observations.

However, if we had assumed that nuclear export and dissociation of HIF-1 is not oxygen-dependent our equations for cytoplasmic and nuclear HIF-1 α would have taken the following form

$$\frac{dx_6}{dt} = c_3 x_1 - c_4 x_6 - k_1(C) x_6 x_9 + k_{-1} x_7 - i_1 x_6 + e_1 x_{12} \quad (4.5)$$

$$\begin{aligned} \frac{dx_{12}}{dt} &= i_1 k_v x_6 - e_1 k_v x_{12} - k_{deg2} x_{12} - p_1(C) x_{12} x_{15} + p_{-1} x_{13} \\ &\quad - a_1 x_{12} x_{18} + a_{-1} x_{19}, \end{aligned} \quad (4.6)$$

and prolyl hydroxylation would be our only oxygen sensing mechanism. These equations can be simplified to

$$\frac{dx_6}{dt} = p_1 - p_2 x_6 - p_3 C x_6 + p_4 x_{12} \quad (4.7)$$

$$\frac{dx_{12}}{dt} = p_5 x_6 - p_4 x_{12} - p_6 x_{12}, \quad (4.8)$$

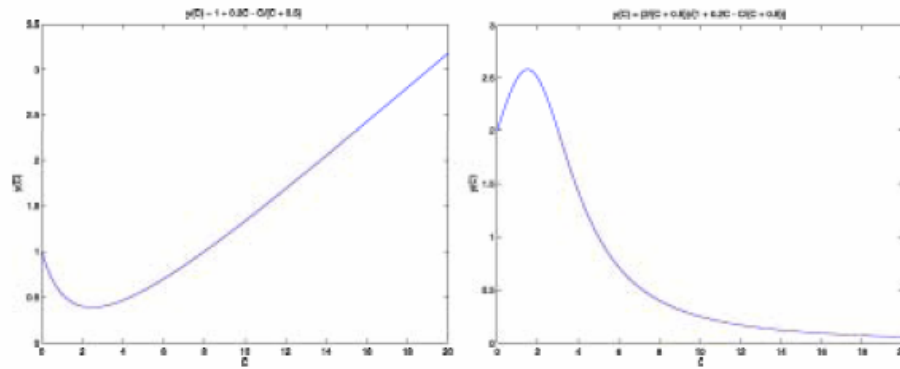


Figure 6: Plot of denominator of nuclear HIF-1 α and plot of nuclear HIF-1 α , as functions of oxygen concentration (C) given in Equation (4.4), where $p_1 = p_2 = p_6 = 1$, $p_3 = 0.2$, $p_4 = 0.5$ and $p_5 = 2$,

and the steady state of the simplified system satisfies

$$x_6 = \frac{p_1}{p_2 + p_3 C - \frac{p_4 p_5}{p_4 + p_6}} \quad (4.9)$$

$$x_{12} = \frac{p_1 p_5 / (p_4 + p_6)}{p_2 + p_3 C - \frac{p_4 p_5}{p_4 + p_6}}. \quad (4.10)$$

We can now plot (Figure 7) the denominator of x_{12} , and x_{12} itself, as a function of oxygen tension, where x_{12} is given by (4.10). We notice that the denominator now only exhibits linear behaviour and subsequently plotting x_{12} ($H_{\alpha N}$) as a function of oxygen concentration gives rise to a monotonically decreasing curve.

We therefore hypothesize that oxygen sensing via proline hydroxylation is an essential part of this model and gives the sharp transitional behaviour as seen between 2% and 0.5% O_2 in the experimental hypoxia response curve published in [34]. However, through the above analysis we suggest that oxygen dependent dissociation and nuclear export may be the mechanisms whereby the hypoxia response starts to decrease in the range 0.5%-0% oxygen.

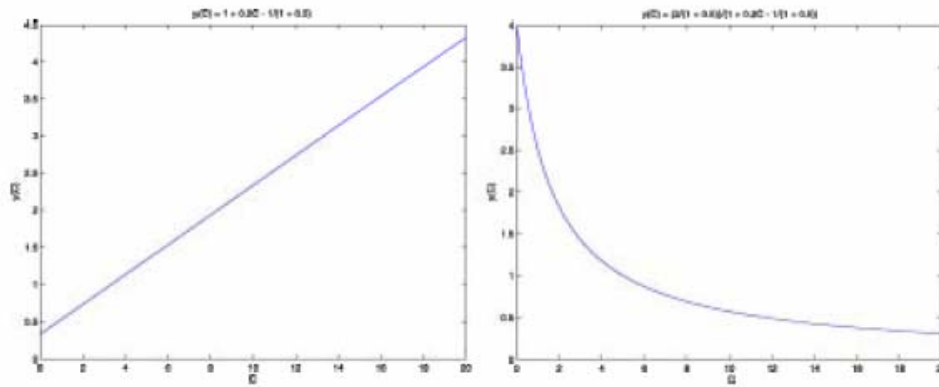


Figure 7: Plot of denominator of nuclear HIF-1 α and plot of nuclear HIF-1 α , as functions of oxygen concentration (C) given in Equation (4.10), where $p_1 = p_2 = p_6 = 1$, $p_3 = 0.2$, $p_4 = 0.5$ and $p_5 = 2$,

5 Results

5.1 Parameter Estimation

One of the biggest problems mathematical modellers face in this area is the lack of biologically determined parameters. To further compound the problem our model is of a large size and therefore consists of a large number of parameters, 54 in fact. Although we have no way of estimating these parameters accurately, we have chosen realistic values for the parameters by taking some estimates from a model of another transcription factor, NF- κ B, by Lipnaicki *et al* [41]. Due to similarities between the HIF-1 α and NF- κ B system we can use their model to estimate the following: mRNA synthesis, mRNA degradation, protein translation, protein degradation and cytoplasmic-nuclear transport. Although the Lipnaicki model describes the regulation of a different set of proteins they have taken the rate constants to be the same for many of the proteins in their model. We therefore feel it is reasonable to apply these estimates to our model proteins. We have chosen the estimates for the first 22 constants $c_1 - c_8$, $c_{14} - c_{17}$, $c_{c9} - c_{c13}$ and $c_{n9} - c_{n13}$ in this way. The other parameter taken from the model directly was k_v , the cytoplasmic to nuclear volume.

We have also used as much of the available experimental data as possible in formulating the rest of our estimates. Firstly it has been reported that HIF-1 α has a half-life of approximately 2 hours in hypoxic conditions and close to 5 minutes in normoxic conditions [14, 22]. In our model the rapid degradation under normoxic conditions is a consequence of the ubiquitination mediated degradation in the proteasome, which is represented through the hydroxylation of HIF-1 α and subsequent interaction with VHL. However, we can take from this that the spontaneous degradation of HIF-1 α corresponds to a half-life of two hours and therefore gives an estimate for k_{deg2} . Secondly, all three prolyl hydroxylases (PHD1, 2 and 3) were shown to have an approximate K_m for oxygen in the region 230-250 μM [21]. PHD2 and 3 have been shown to be the most effective of the three hydroxylases and both have a K_m for oxygen of 250 μM . We have therefore taken $K_m = 250\mu M$ for our generic prolyl hydroxylases and used this to fit k_1 , k_{-1} and k_2 . We then set $p_1 = p_4 = k_1$, $p_{-1} = p_{-4} = k_{-1}$ and $p_2 = p_5 = k_2$. We list all our model parameters in Table 2 with a description of the parameter, corresponding unit of measurement and parameter value used in our model simulations.

5.2 Simulation Results

To approximate the solution of our model, we used the Matlab ODE solver ODE45. The time and space steps employed in the finite difference scheme are decided automatically by the solver. We ran the simulations using the initial values in Table 1 for a time $T=250$ seconds and for a range of values of oxygen tension between 0-20%. In Figure 8 we have plotted the final time values for total nuclear HIF-1 α ($H_{\alpha N}(t) + H_{\alpha NP}(t)$) and total occupied HRE ($HRE^I(t) + HRE^*(t)$) against oxygen tension (C).

Our model simulation shown in Figure 8 can be compared directly to the experimental hypoxia response curve by Jiang *et al* [34], which has been schematically reproduced in Figure 3(a). The data points may not match exactly but this is not to be expected since the parameters have only been roughly estimated. Our goal was to capture the qualitative behaviour

Parameter name	Units	Description	Estimated Value	Reference
σ_1	$\text{mM}^{-1} s^{-1}$	HIF-1 α -constitutive mRNA transcript synthesis	5×10^{-7}	Lipnacki et al
σ_2	s^{-1}	HIF-1 α mRNA degradation	0.0004	Lipnacki et al
σ_3	s^{-1}	HIF-1 α translation rate	0.5	Lipnacki et al
σ_4	s^{-1}	Spontaneous HIF-1 α protein degradation	0.0007	Assumption
σ_5	$\text{mM}^{-1} s^{-1}$	HIF-1 β -constitutive mRNA transcript synthesis	5×10^{-7}	Lipnacki et al
σ_6	s^{-1}	HIF-1 β mRNA degradation	0.0004	Lipnacki et al
σ_7	s^{-1}	HIF-1 β translation rate	0.5	Lipnacki et al
σ_8	s^{-1}	HIF-1 β protein degradation	0.0007	Assumption
σ_9	$\text{mM}^{-1} s^{-1}$	PHD1-constitutive mRNA transcript synthesis	5×10^{-7}	Lipnacki et al
σ_{10}	s^{-1}	PHD1 HIF-inducible mRNA synthesis	0	Lipnacki et al
σ_{11}	s^{-1}	PHD1 mRNA degradation	0.0004	Lipnacki et al
σ_{12}	s^{-1}	PHD1 translation rate	0.5	Lipnacki et al
σ_{13}	s^{-1}	Spontaneous PHD1 protein degradation	0.0007	Assumption
σ_{14}	$\text{mM}^{-1} s^{-1}$	VHL-constitutive mRNA transcript synthesis	5×10^{-7}	Lipnacki et al
σ_{15}	s^{-1}	VHL mRNA degradation	0.0004	Lipnacki et al
σ_{16}	s^{-1}	VHL translation rate	0.5	Lipnacki et al
σ_{17}	s^{-1}	Spontaneous VHL protein degradation	0.0007	Assumption
k_1	$\text{mM}^{-2} s^{-1}$	Cytoplasmic HIF-1 α -PHDc association	0.01	Fitted (from PHD K_m for O_2)
k_{-1}	s^{-1}	Cytoplasmic HIF-1 α -PHDc dissociation	0.001	Fitted (from PHD K_m for O_2)
k_2	s^{-1}	Formation of cytoplasmic hydroxylated HIF 1 α	2.5	Fitted (from PHD K_m for O_2)
k_3	$\text{mM}^{-1} s^{-1}$	Cytoplasmic hydroxylated HIF-1 α -VHL association	0.05	Assumption
k_{-3}	s^{-1}	Cytoplasmic hydroxylated HIF-1 α -VHL dissociation	0.001	Assumption
p_1	$\text{mM}^{-2} s^{-1}$	Nuclear HIF-1 α -PHDn association	0.01	$= k_1$
p_{-1}	s^{-1}	Nuclear HIF-1 α -PHDn dissociation	0.001	$= k_{-1}$
p_2	s^{-1}	Formation of nuclear hydroxylated HIF 1 α	2.5	$= k_2$
p_3	$\text{mM}^{-1} s^{-1}$	Nuclear hydroxylated HIF-1 α -VHL association	0.05	$= k_3$
p_{-3}	s^{-1}	Nuclear hydroxylated HIF-1 α -VHL dissociation	0.001	$= k_{-3}$
p_4	$\text{mM}^{-2} s^{-1}$	Nuclear HIF-1 α -PHDn association	0.01	$= k_1$
p_{-4}	s^{-1}	Nuclear HIF-1 α -PHDn dissociation	0.001	$= k_{-1}$
p_5	s^{-1}	Formation of nuclear hydroxylated HIF 1 complex	2.5	$= k_2$
α_1	$\text{mM}^{-1} s^{-1}$	HIF-1 α -HIF-1 β association	0.008	Assumption
α_{-1}	$\text{mM}^{-1} s^{-1}$	HIF-1 α -HIF-1 β dissociation	0.07	Assumption
α_2	$\text{mM}^{-1} s^{-1}$	HIF 1 HRE association	0.5	Assumption
α_{-2}	$\text{mM}^{-1} s^{-1}$	HIF-1-HRE dissociation	0.8	Assumption
α_3	$\text{mM}^{-1} s^{-1}$	Hydroxylated HIF-1 α -HIF-1 β association	0.008	$= \alpha_1$
α_{-3}	s^{-1}	Hydroxylated HIF-1 α -HIF-1 β dissociation	0.07	$= \alpha_{-1}$
α_4	$\text{mM}^{-1} s^{-1}$	Hydroxylated HIF-1-HRE association	0.5	$= \alpha_2$
α_{-4}	s^{-1}	Hydroxylated HIF-1-HRE dissociation	0.8	$= \alpha_{-2}$
i_1	s^{-1}	HIF-1 α nuclear import	0.0001	Assumption
e_1	$\text{mM}^{-1} s^{-1}$	HIF-1 α nuclear export	8×10^{-6}	Assumption
i_2	s^{-1}	Hydroxylated HIF-1 α nuclear import	5×10^{-5}	Assumption
e_2	s^{-1}	Hydroxylated HIF-1 α nuclear export	0.008	Assumption
i_4	s^{-1}	VHL nuclear import	2.5	Assumption
e_4	s^{-1}	VHL nuclear export	3.5	Assumption
k_{deg}	s^{-1}	Hydroxylated HIF-1 α -VHL degradation	2.5	Assumption
p_{deg}	s^{-1}	Nuclear hydroxylated HIF-1 α -VHL degradation	2.5	$= k_{deg}$
k_{deg2}	s^{-1}	Spontaneous nuclear HIF 1 α protein degradation	6×10^{-6}	Fitted (from HIF-1 α half-life)
k_{deg3}	s^{-1}	Degradation/removal of Occupied HRE	0.03	Assumption
k_v		Nuclear/Cytoplasmic volume	5	Lipnacki et al

Table 2: Table of parameters and units

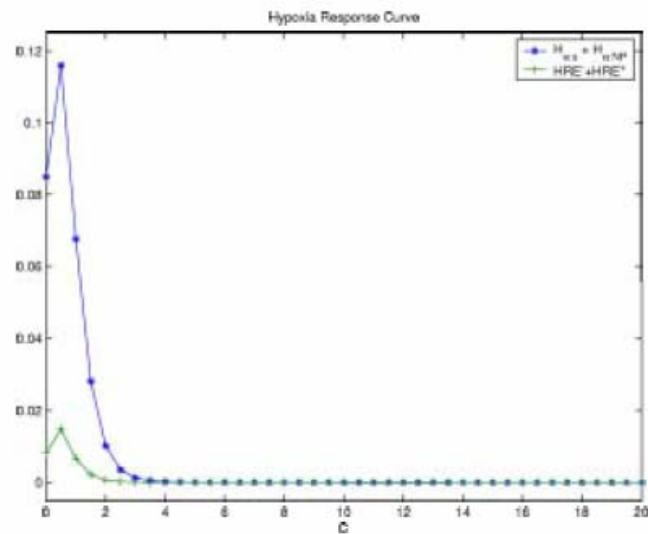


Figure 8: Plot of Nuclear HIF-1 α and Occupied HRE against oxygen tension from model Equations (3.14), (3.16), (3.25) and (3.26).

of the hypoxia response and then highlight the mechanisms that caused it.

In this sense our simulation compares very well with the experimental hypoxia response curve because we capture the switch-like response between 0.5 and 2% oxygen, with the maximal response occurring at 0.5% O₂. We also manage to capture the decreasing response as oxygen tension approaches 0%.

6 Discussion

In this paper we have extended a model by Kohn et al [1] which describes the cellular response to hypoxia that is mediated by the transcription factor HIF-1. The Kohn model consisted of a system of twenty ODEs, and successfully replicated a number of experimental observations. The model did not, however, fully capture all the qualitative behaviour of the oxygen dose-response curve. We have proposed an alternative model with more dependance on oxygen. We have

shown using numerical and analytic techniques that the new model can capture all important features of the dose-response curve. Unfortunately however, our model fails to reproduce an experimental result concerning VHL as well as Kohn's original model.

One of the main reasons that HIF-1 has been thrown into the spotlight is because of its involvement in clear-cell renal carcinoma and other VHL deficient tumours. VHL has been implicated as an important feature in the HIF-1 α degradation pathway due to binding with the hydroxylated form of HIF-1 α , thereby targeting it for ubiquitination and degradation by the proteasome. In VHL deficient tumours HIF-1 α and HIF-1 target genes are constitutively expressed resulting in abnormally high vascularisation. Using their model, Kohn *et al* [1] performed numerous simulations, including one in which they measured the total HRE occupancy for varying amounts of VHL in the system. The results successfully reproduced the observation that HRE occupancy is increased under normoxic conditions as total VHL is decreased. Furthermore they were able to reproduce constitutive HRE occupancy when VHL is completely absent.

In the process of extending the Kohn model we have made certain changes such that our new model cannot reproduce these clinical results as well as the model of Kohn *et al*. One of our future aims is to analyse why.

6.1 Future directions

Using a mathematical model we have been able to suggest why certain proposed mechanisms within the HIF system are not alone sufficient to give the experimentally observed behaviour. We have also suggested somewhat original mechanisms that are crucial to the system. Below we list the predictions made by the model:

1. Nuclear export of HIF-1 α on reoxygenation is oxygen dependent and an important mechanism in the system.
2. There is some negative regulation of HIF-1 binding and binding of HIF-1 to hypoxia response elements.

3. There is some degradation of HIF-1/HIF-1 α succeeding transcriptional activation.

We concede the main flaws in our model; firstly that our model is too large with too many parameters and secondly that the majority of our parameters are not experimentally derived. Our future goals are to reduce the model down to a manageable size. We aim to approach this problem from two different angles, (i) using scaling arguments to reduce the system to a lower order ODE system being solved on a manifold, and (ii) simplifying the biochemical network diagram from the outset to derive a caricature model which keeps the relevant features that we need for the problem we are trying to solve. This may allow us to direct experimentalists into measuring the subset of parameters that are crucial to the behaviour of the system.

There are many ways in which we could extend or improve the model. For instance, we have only included one of the five pathways regulating HIF function. We would like to extend the model to include asparagine hydroxylation and the effect of transcription repression. We would then like to go on to consider the effect of the three remaining pathways that have been discovered more recently. We would also like to extend the model to include the downstream pathways such as angiogenesis, glycolysis and apoptosis in order to test the effect of therapeutic strategies such as knocking out HIF-1. However, we must be careful that we do not end up with a huge complicated model which does not allow us to gain insight into the system. To avoid this pitfall it is necessary that we first carry out the type of model reduction described above.

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